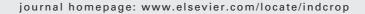
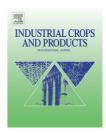


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Functional properties of protein from Lesquerella fendleri seed and press cake from oil processing

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ABSTRACT

This investigation determined the functional properties of protein in Lesquerella fendleri seed and press cake from oil processing. L. fendleri seeds were heat-treated at 82 °C (180 °F) during 120 min residence time in the seed conditioner, and then screw-pressed to extract the oil. Unprocessed ground, defatted lesquerella seeds and press cakes were analyzed for proximate composition and protein functional properties. Protein from unprocessed lesquerella seed showed the greatest solubility (\geq 60%) at pH 2 and 10 and was least soluble (25%) at pH 5.5–7. Unprocessed lesquerella protein also had high surface hydrophobicity index (S_0), as well as, excellent foaming capacity and stability, emulsifying properties, and water-holding capacity (WHC) at pH 7. Protein solubility profile of the press cake showed up to 50% reduction in soluble proteins at nearly all pH levels, indicating heat denaturation during cooking and screw-pressing. Foaming capacity of the press cake protein decreased slightly, but foam stability was completely lost. Press cake protein also had markedly reduced values for S_0 , emulsifying properties and WHC, further confirming lesquerella protein's sensitivity to heat treatment.

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1. Introduction

The genus Lesquerella comes from the same family, Brassicaceae, as rapeseed, canola, and mustard. This plant originated in South and North America, and 83 of the more than 100 known species occur in the northern American region (Dierig et al., 1992). One species, Lesquerella fendleri (S. Wats.), has been found to have the most productive seed yield both in the wild and under cultivation and is the only species being domesticated (Carlson et al., 1990; Dierig et al., 1992). L. fendleri is native to Arizona, New Mexico, Colorado, Utah, Texas, and Mexico. The primary reason for the interest in lesquerella is its oil. Depending on the region of the country where the crop is grown, lesquerella oil contains pre-

dominantly lesquerolic, densipolic, or auricolic hydroxy acids, which are hydroxy FA with structures that are similar to ricinoleic acid, the main FA in castor oil (Dierig et al., 1992; Abbott et al., 1997). Lesquerella oil is regarded as a domestic alternative to imported castor oil for use in high-performance lubricants, paints, plastics, pharmaceuticals and cosmetics (Abbott et al., 1997). If lesquerella oil is commercialized successfully, we expect that protein-rich meals will be major co-products of the process.

L. fendleri seed has 22% crude protein (dry basis, db) (Miller et al., 1962; Carlson et al., 1990). Earlier studies found that the meal contained favorable amounts of lysine, methionine, and threonine, suggesting that the meal would be a good supplement for feed grains (Miller et al., 1962; Carlson et al., 1990).

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Little else is known about L. fendleri seed protein. In contrast, the literature provides several studies on the production, composition, and functionality of rapeseed and canola proteins. Researchers found that, in general, rapeseed and canola proteins possessed functional properties that were comparable to those of soybean protein (Ohlson and Anjou, 1979; Dev and Mukherjee, 1986; McCurdy, 1990; Xu and Diosady, 1994) or egg white (Thompson et al., 1982). Until recently, researchers interested in lesquerella would have relied on studies involving these related crops to infer the possible functional properties of lesquerella protein. We believe that our latest findings on the fractionation and characterization of L. fendleri protein (Wu and Hojilla-Evangelista, 2005) were the first to be reported on this subject. We determined that L. fendleri protein was made up predominantly of salt-soluble and NaOH-soluble fractions (24% and 20%, respectively, of the total protein), while the water-soluble and ethanol-soluble fractions were not significant (10% and 1%, respectively, of the total protein). We detected 11 distinct bands (M.W. 6-100 kDa) from the reduced proteins in the whole seed, with the dominant sub-units resolving at approximately 53, 36, 22, and 6kDa. We noted that L. fendleri meal contained high amounts of glutamic acid, lysine, cysteine, and methionine at levels that were greater than those found in cereal and soy protein and thus, can meet the nutritional requirements of children and adults.

More information about the quality and properties of lesquerella protein is needed to optimize its use when evaluating its potential applications. The present study was done to determine the functional properties of proteins in *L. fendleri* seeds. Additionally, the study evaluated the effects of heating during oil extraction on the functionality of proteins in *L. fendleri* press cake to establish the extent of heat-induced protein denaturation that was anticipated to occur.

2. Materials and methods

2.1. Starting materials

L. fendleri seeds were obtained from the 2003 crop grown in Arizona. Seeds were cleaned by screening and aspiration. Cleaned whole seeds were then placed in the laboratory seed conditioner (Model 324, French Oil Mill Machinery Co., Piqua, OH) set at 82 °C (180 °F) in the top deck and 104 °C (220 °F) in the bottom deck. These conditions allowed the seeds to have 120 min of residence time; i.e., time to bring the seed to cooking temperature (ca. 50 min) + cooking time (ca. 60 min) + drying time of the seed. Cooking for 60 min at 212 °F was reported to be sufficient to inactivate the thioglucosidase in L. fendleri seeds having 6% MC (Carlson et al., 1990). Oil was extracted from the cooked whole seeds by using a heavy-duty laboratory screw-press (Model L-250, French Oil Mill Machinery Co., Piqua, OH). Press cakes were recovered for composition and protein functionality analyses.

2.2. Proximate analyses

Unprocessed L. fendleri seeds and press cakes from oil processing were ground into ca. 30-mesh particle size by using a Cuisinart coffee grinder (Model DCG-12BC, East Windsor, NJ)

for 2 min. Moisture, crude protein ($\%N \times 6.25$), and crude oil contents of the samples were determined by using AOCS standard methods Ba 2a-38, Ba 4e-93, and Ba 3-38, respectively (AOCS, 1998).

2.3. Native gel electrophoresis

Electrophoresis of native proteins was done according to the procedure we reported previously for Cuphea (Hojilla-Evangelista and Evangelista, 2006). Ground, unprocessed L. fendleri seeds and press cakes were weighed into centrifuge tubes to provide $4\,mg\,protein/mL$ in $500\,\mu L$ of commercial native sample buffer (Invitrogen Tris-glycine native sample buffer, pH 8.6, Invitrogen Corp., Carlsbad, CA). The tubes were shaken for 10 min on a platform shaker and then centrifuged at 5000 \times g for 5 min. Twenty μ L of supernatants were loaded onto pre-cast NovexTM Tris-glycine 6–18% gradient gel. Unstained protein standards (Invitrogen NativeMarkTM, M.W. from 20 to 1236 kDa) were included in the gel. Electrophoresis was done at 125 V for 78 min in an Invitrogen XCell SureLock $^{\text{TM}}$ Mini-Cell system. The running buffer was Invitrogen Tris-glycine native running buffer (25 mM Tris base, 192 mM glycine), pH 8.3, which was diluted to 10× volume with nanopure water before use.

2.4. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was done by following the method of Wu and Hojilla-Evangelista (2005). Ground *L. fendleri* seeds and press cakes were weighed out to provide 4 mg protein/mL in 500 μ L of sample buffer that contained 42 mM Tris–HCl (pH 6.8), 2% SDS, 7% glycerol, 4.4% β -mercaptoethanol, and 5 M urea. The mixture was then heated in a boiling-water bath for 5 min. Protein samples (15 or 8 μ L) were loaded onto 4–12% Bis-Tris NuPAGE pre-cast gradient gel (Invitrogen Corp., Carlsbad, CA). Bio-Rad (Bio-Rad Laboratories, Hercules, CA) pre-stained broad range SDS-PAGE protein standards (6.5–196 kDa) were included in the gel. Electrophoresis was done in a Novex XCell II Mini Cell system (Novex, San Diego, CA) and using the NuPAGE MES-SDS running buffer (SDS, Tris, and 4-morpholinoethane sulfonic acid).

2.5. Functionality tests

Oil from ground *L. fendler*i seeds and press cakes were first extracted by using hexane at 25 °C according to the procedure we followed for *Cuphea* (Hojilla-Evangelista and Evangelista, 2006). This method permitted the evaluation of properties of the protein while in its native state and adequately eliminated the effects of oils on functional properties of protein as long as the residual oil content was \leq 0.5% (db). Four extraction cycles were done to obtain residual oil contents of \leq 0.5% (db). In each extraction, the mixture (1g sample:10 mL solvent) was stirred for 1h with a magnetic bar. The mixture was allowed to stand until the supernatant has cleared, and then the solvent layer was pipetted out and discarded. Defatted ground samples were air-dried in a fume hood until the hexane smell was no longer detectable, and then stored in screw-capped polyethylene containers at room temperature until use.

(% db) Crude protein (% db, ffbb)							
Before hexane-defatting							
b $30.3 \pm 1.5a$							
a $32.3 \pm 0.2a$							
After hexane-defatting							
a $28.8 \pm 0.7a$							
a 31.9 \pm 0.3a							
).7).3							

Values are means \pm standard deviations of duplicate determinations. In a sample block (before or after defatting), means within a column followed by different letters (a and b) are significantly different (p < 0.05).

2.5.1. Protein solubility

Protein solubilities of samples containing 10 mg protein/mL were determined at pH 2.0, 4.0, 5.5, 7.0, 8.5, and 10.0 according to the method of Balmaceda et al. (1984). The yellow to deep amber supernatants were freeze-dried and their Dumas N contents were determined by AOCS method Ba 4e-93 (AOCS, 1998).

2.5.2. Surface hydrophobicity

Surface hydrophobicity indices (So) of soluble proteins in the extracts were determined at pH 7.0 and 10.0 as described by Hojilla-Evangelista et al. (2004), which was adapted from the method of Sorgentini et al. (1995). Samples were weighed out to provide 2 mg protein/mL and dispersed in 0.01 M phosphate buffer (pH 7) or 0.025 M NaHCO₃ • Na₂CO₃ buffer (pH 10). Supernatants were diluted with the pH 7 or pH 10 buffer to yield 1/5, 1/10, 1/50, 1/100, and 1/500 concentrations of the starting protein content. The fluorescence probe was 8.0 mM 8-anilino-1-naphthalene sulfonate (ANS). Fluorescence intensities (FI) were measured by a Varian Cary Eclipse Fluorescence Spectrophotometer (Walnut Creek, CA) at a slit opening of 10 nm and wavelengths of 350 nm (excitation) and 525 nm (emission). FI values were plotted against protein concentrations to determine So, which corresponded to the initial slope of the graph as calculated by linear regression.

2.5.3. Emulsifying properties

Emulsifying properties were determined according to the method of Wu et al. (1998). Samples were weighed out to provide 1 mg protein/mL and dispersed in 0.01 M phosphate buffer (pH 7) or 0.025 M NaHCO₃•Na₂CO₃ buffer (pH 10). Small amounts of 0.1 M NaOH were added to attain a final sample pH of 7.0 or 10.0. Mixtures were allowed to stand for 15 min. Emulsions were prepared by homogenizing mixtures of 6 mL sample supernatants and 2 mL corn oil with a hand-held homogenizer operated at high setting (20,000 rpm) for 1 min. Emulsification activity index (EAI, in m²/g) and emulsion stability index (ESI, in min) were calculated from absorbance readings taken at 500 nm.

2.5.4. Foaming properties

Foaming properties were determined according to the procedure of Myers et al. (1994), which we modified to take into account the capacity of ground *L. fendleri* samples to absorb substantial amounts of water. Forty milliliters of samples

containing 10 mg protein/mL were prepared to ensure that sufficient volume of supernatant will be recovered for the foaming tests. Their pHs were immediately adjusted to 7.0 or 10.0 by addition of 0.1 M NaOH. Samples were centrifuged at $3650 \times g$ and $25\,^{\circ}\text{C}$ for 30 min, and the supernatants were then subjected to foaming tests. Foam capacity was the volume (mL) of foam produced in 1 min. Foam stability was the fraction of foam volume remaining (%) after standing for 15 min.

2.5.5. Water-holding capacity (WHC)

WHC of the samples was determined by adapting the method of Balmaceda et al. (1984) for insoluble or partly soluble materials. We modified the method in the following ways: capped tubes with samples were placed on a platform shaker for 15 min instead of being stirred individually, sample pH was adjusted to 7.0 or 10.0 by adding 0.1 M NaOH, and a water-bath set at 60 $^{\circ}$ C was used for heating instead of a hot plate. All other steps and calculations were done exactly as described in the original method.

2.6. Statistical analyses

Statistical analyses were performed by using the SAS® Systems for Windows software (SAS Institute Inc., Cary, NC). Analysis of variance and Bonferroni t-tests or Duncan's Multiple Range tests were performed on duplicate replications of data to determine significant differences among the treatments (p < 0.05).

3. Results and discussion

3.1. Moisture, oil and protein contents

Previous studies have reported that *L. fendleri* seed contains 22–26% oil, 22–29% protein (<1% non-protein nitrogen), 13% crude fiber, 6.5% ash, and 49% carbohydrates and other nitrogen-free components (Miller et al., 1962; Carlson et al., 1990). Oil content of our unprocessed *L. fendleri* seed (Table 1) was slightly less than the 26–28% (db) reported by Miller et al. (1962) and Carlson et al. (1990). This amount was only one-half of the oil content of rapeseed (Ohlson and Anjou, 1979). Screw-pressing substantially reduced oil content in the resulting press cake. Additional defatting with hexane further decreased residual oil contents to less than 1% in both seed and press cake. *L. fendleri* seed contained about 30% crude pro-

^a Dumas N × 6.25.

^b ffb means fat-free basis.

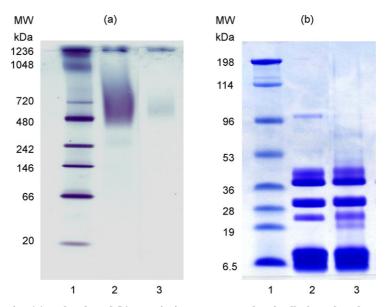


Fig. 1 – Band patterns of native (a) and reduced (b) protein in unprocessed L. fendleri seed and press cake: (1) M.W. standards; (2) ground, defatted seed; (3) press cake. Concentration = 4 mg protein/mL; sample load volume = $20 \mu L$ in native gel, $15 \mu L$ in reducing gel.

tein (moisture-free and fat-free basis) (Table 1), which was the same as those determined by Miller et al. (1962) and Carlson et al. (1990). Oil processing had no apparent effect on the crude protein content of *L. fendleri*, as indicated by similar values (moisture-free and fat-free basis) for the unprocessed seed and press cake (Table 1).

3.2. Electrophoresis results

The native protein in unprocessed L. fendleri seed showed three bands that resolved near 242 kDa, between 480 and 800 kDa, and 1236 kDa (Fig. 1a, lane 2). The dominant sub-unit, indicated by the darkest color, resolved between 480 and 800 kDa and its high M.W. may indicate a cross-linked structure. The native protein in the press cake (lane 3) showed only two bands that appeared near 720 and 1236 kDa. These bands were much lighter colored, narrower, and less defined, which indicated that L. fendleri protein was sensitive to the heat applied during oil processing. Thermal treatments of proteins often result in structural changes and hydrolysis of peptide bonds, which, in turn, greatly influence protein functionality (Cheftel et al., 1985). The effect of heating was not obvious in the reduced proteins, as both unprocessed L. fendleri seed and press cake showed very similar band patterns (Fig. 1b). There were eight visible bands on the SDS-PAGE gel loaded with reduced proteins, all resolving at M.W. less than 100 kDa. The darkest bands resolved at ca. 45, 40, 30, 25, 10, and <6 kDa. These band patterns agreed with what we observed previously for reduced protein in L. fendleri meal (Wu and Hojilla-Evangelista, 2005).

3.3. Protein solubility

The protein solubility profile of L. fendleri seed (Fig. 2) had a similar shape to that of rapeseed flour protein obtained by Thompson et al. (1982). L. fendleri seed protein was most

soluble (60–65%) at pH 2.0 and pH 10.0. The increased protein solubility at alkaline pH supported our earlier finding for the meal (Wu and Hojilla-Evangelista, 2005). The presence of alkali generally improves protein solubility by causing dissociation and disaggregation of proteins; however, high solubility at alkaline pH may also be caused by extensive proteolysis (Kinsella, 1976).

L. fendleri seed protein was least soluble (25%) at pH 5.5–7.0 (Fig. 2). This was contrary to what we observed previously for the defatted meal (Wu and Hojilla-Evangelista, 2005), which exhibited the lowest nitrogen solubility (15%) near pH 4.2. Thompson et al. (1982) also reported minimal protein solubility (29%) at pH 4 for rapeseed flour. However, Radwan and Lu (1976) found that rapeseed meal showed the least nitrogen solubility (14–19%) at pH 4.2–7.0, a range that included what we noted for L. fendleri seed in the present study.

There were significant reductions in the amounts of soluble proteins in *L. fendleri* press cake (Fig. 2) at nearly all pH lev-

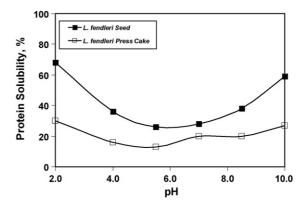


Fig. 2 – Protein solubility profiles of ground, defatted L. fendleri seed and press cake from whole seed cooked for 120 min in the seed conditioner.

Table 2 – Functional properties of ground L. fendleri seeds and press cake at pH 7 and pH 10.								
Sample and pH		Functional properties ^a						
	S _o	EAI (m²/g)	ESI (min)	FC (mL)	FS (% foam left)	WHC (g water/g protein)		
L. fendleri seed, pH 7	923 ± 23a	93.4 ± 2.2a	25.1 ± 2.6a	$151 \pm 4a$	79.5 ± 6.4a	8.05 ± 0.85a		
L. fendleri seed, pH 10	$721 \pm 7b$	$64.9 \pm 3.5b$	$13.7\pm0.3b$	$132\pm0b$	$64.8 \pm 5.6a$	$8.36 \pm 0.28a$		
Press cake, pH 7	$530\pm14d$	$32.2\pm5.8d$	$14.8\pm2.9b$	$116 \pm 7c$	$0.0\pm0.0b$	$5.99 \pm 0.71b$		
Press cake, pH 10	643 ± 5c	47.0 ± 1.7c	17.6 ± 0.9b	112 ± 1c	0.0 ± 0.0b	5.97 ± 0.32b		

Values are means \pm standard deviations of duplicate determinations. Means within a column followed by different letters (a–d) are significantly different (p < 0.05).

els, with the most pronounced decrease observed at extremely acidic and basic pH values. The smallest reduction in protein solubility occurred at pH 7.0. These findings showed that heat applied during oil processing had deleterious effects on the solubility of *L. fendleri* seed protein, which corroborated our results from native gel electrophoresis (Fig. 1a).

The solubility profile of a protein is a practical indicator of its denaturation and also gauges the potential or limitation of the protein as a functional ingredient (Thompson et al., 1982) because it is related to other properties, such as foaming, emulsification, and gelation. *L. fendleri* seed protein showed high solubility at pH 10 and still had substantial solubility at pH \leq 7, which implied that the protein may be useful in highly alkaline, as well as, aqueous systems. Thus, the other functional properties were evaluated at both neutral pH and pH 10.

3.4. Surface hydrophobicity index (So)

So is an indicator of the extent of exposure of hydrophobic regions of protein molecules; higher values suggest more unaggregated proteins. The So for unprocessed L. fendleri seed protein determined at neutral pH was slightly greater than that obtained at pH 10 (Table 2) and almost two times greater than the 530 we calculated as So for soybean protein (Hojilla-Evangelista et al., 2004). This result suggests that at neutral pH, L. fendleri seed proteins are better dispersed or less aggregated. So values for the press cake protein were much less at pH 7 than at pH 10, compared to those of the unprocessed seed. These markedly lower So values implied a greater presence of aggregated proteins or degree of denaturation in the press cake, which may be traced again to the deleterious effect of heat on the protein. We are unable to make further comparisons involving our lesquerella So results as there are no previous reports on protein functionality of this crop. Additionally, although there have been studies on the protein functional properties of the closely related crops rapeseed and canola (Thompson et al., 1982; Dev and Mukherjee, 1986; Xu and Diosady, 1994), none of them evaluated surface hydrophobicity.

3.5. Emulsifying properties

The emulsification activity index (EAI) is a measure of a protein's emulsifying capacity, with higher EAI values indi-

cating better emulsifying capacity. The emulsifying capacity of unprocessed *L. fendleri* seed protein was far superior at pH 7 than at pH 10 (Table 2), as shown by an EAI value that was greater than what was determined at the alkaline pH. The EAI at pH 7 for *L. fendleri* protein was also much higher than that of soybean protein (56 m²/g protein) (Hojilla-Evangelista et al., 2004). Rapeseed protein, on the other hand, has been reported as having an emulsifying capacity that was comparable to that of soybean protein (Thompson et al., 1982; Dev and Mukherjee, 1986). In contrast, Xu and Diosady (1994) found that soybean protein had better emulsifying activity than either rapeseed or canola meal.

L. fendleri press cake had EAI values that were significantly less than those recorded for unprocessed seed at the two pH settings (Table 2), which strongly demonstrated the detrimental effect of heat on the protein's emulsifying ability. Thermal treatments can result in improved emulsifying capacity if the protein structure can unfold without resulting in aggregation (Cheftel et al., 1985).

Emulsion stability index (ESI) results showed that the emulsion from unprocessed L. fendleri seed protein was more stable at pH 7 than at pH 10 (Table 2). ESI at pH 7 for L. fendleri protein was nearly two times greater than that of soybean protein (Hojilla-Evangelista et al., 2004). In comparison, Xu and Diosady (1994) reported similar emulsion stabilities among proteins from soybean, canola, and rapeseed, while Dev and Mukherjee (1986) found that rapeseed protein had poorer emulsion stability than soybean protein. L. fendleri press cake had ESI values that were similar to that of the unprocessed seed at pH 10. At pH 7, cooking and screw-pressing markedly reduced the ESI value (Table 2), which suggested that lesquerella protein subjected to prior heating formed less stable emulsions. The impaired emulsion stability may have been the result of reduced protein solubility in the press cake, such that there were not enough soluble proteins that could adsorb at the oil-water interface to lower the interfacial tension and prevent coalescence (Cheftel et al., 1985).

3.6. Foaming properties

As with the preceding functional properties, unprocessed *L. fendleri* seed protein produced greater foam volume at pH 7 than at pH 10 (Table 2). The foaming capacity at pH 10 was nearly identical to the 131 mL of foam produced by soybean protein at neutral pH (Hojilla-Evangelista et al., 2004). *L. fend-*

^a S_o, Surface hydrophobicity index; EAI, emulsion activity index; ESI, emulsion stability index; FC, foaming capacity; FS, foam stability; WHC, water-holding capacity.

leri protein's excellent foaming capacity was consistent with the findings of Dev and Mukherjee (1986) on rapeseed protein products, which they found to have better foaming capacity than did soybean protein. Xu and Diosady (1994) reported that rapeseed and canola meals had foaming capacities that were similar to that of soybean protein. Foams produced by unprocessed *L. fendleri* seed protein were also stable. At neutral pH, 80% of the foam remained after standing for 15 min while at pH 10, two-thirds of the foam was retained after the allotted time. These values for foam stability, however, were less than the 95% remaining foam observed for soybean protein at pH 7 (Hojilla-Evangelista et al., 2004). In contrast, foam stability of rapeseed protein was found to be better than or at least similar to that of soybean protein (Dev and Mukherjee, 1986; Xu and Diosady, 1994).

Protein from *L. fendleri* press cake produced substantially less foam volume at both pH settings than did the unprocessed seed and also completely lost its foam stability (Table 2), as the foams collapsed immediately after being formed. These results provided further compelling evidence of the detrimental effects of heating on lesquerella protein. We postulated in the preceding discussions that heating probably caused protein aggregation, which, in this case, may have limited the availability of proteins that can participate in interfacial interactions, resulting in poorer foam capacity and stability of the press cake protein.

3.7. Water-holding capacity (WHC)

Unprocessed L. fendleri seed protein had WHC values (Table 2) that were markedly greater than the 6.70 gwater/gprotein determined at pH 7 for soybean protein (Hojilla-Evangelista et al., 2004). L. fendleri seeds have been found to contain 19.5% gums (Tookey et al., 1962) and we believe that lesquerella protein's WHC was probably enhanced by the presence of coextracted gums. Further studies are needed to determine the contribution of gums to lesquerella's water-binding properties. WHC values for the press cake were practically identical at the two pHs used, but both were notably less than those obtained for unprocessed L. fendleri seed (Table 2). The lower WHC of the press cake protein may be attributed to the thermal treatment applied during cooking and screw-pressing. Heating typically lessens protein WHC, because the resulting denaturation and aggregation reduce the availability of polar amino groups for hydrogen bonding with water molecules (Cheftel et al., 1985).

4. Conclusions

L. fendleri seed protein displayed noteworthy functional properties at pH 7 and pH 10, which strongly suggested that protein from this new oilseed crop may be used in a variety of applications. Press cake protein gave consistently lower values than the unprocessed seed in all the functionality tests, clear indications of the significant detrimental effects of heating during cooking and screw-pressing. The impaired functional properties of the L. fendleri press cake protein will likely limit its usefulness in food-based systems. However, its relatively good solubility at alkaline pH may be exploited in non-food appli-

cations, such as glues, particularly when combined with its inherent gums.

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